Guidance for Industry

Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

DRAFT GUIDANCE

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GUIDANCE FOR INDUSTRY[1]

Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors

I. INTRODUCTION

This guidance document applies to the manufacture of gene therapy retroviral vector products intended for in vivo or ex vivo use and to follow-up monitoring of patients who have received retroviral vector products. Guidance is provided for replication competent retrovirus (RCR) testing during manufacture, including timing, amount of material to be tested, and general testing methods. In addition, guidance is provided on monitoring patients for evidence of retroviral infection. This guidance document is intended to supplement the guidance and recommendations pertaining to RCR testing given in the following documents: 1) "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy," March 1998; and 2) a letter to Sponsors of INDs Using Retroviral Vectors, dated September 20, 1993. For general guidance on gene therapy refer to "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy" March 1998.

II. BACKGROUND

CBER's current recommendations for RCR testing during retroviral vector production and patient monitoring were developed in 1993, at a time when clinical experience with retroviral vectors was limited (4). The overriding safety issues associated with the use of retroviral vectors are exemplified by the findings of an experiment involving administration of ex vivo transduced bone marrow progenitor cells that had been inadvertently exposed to high titer RCR contained in the retroviral vector material to severely immunosuppressed Rhesus monkeys. In this setting, 3/10 animals developed lymphomas and died within 200 days (3). The RCR was presumed to be etiologically associated with the disease by virtue of the presence of multiple

[1] This draft guidance document represents the agency's current thinking regarding testing for replication competent retrovirus in retroviral vector based gene therapy products. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

murine RCR sequences in the monkey lymphomas and the observed correlation between the lack of antiretroviral antibody response and the development of prolonged retroviremia and disease (9, 11). Since 1993, accumulating experience with different vector producing cells, RCR detection assays and results from patient monitoring have allowed the generation of a small data base of information on the safety of the use of retroviral vectors in clinical applications of gene therapy. This information base has provided a framework for discussion of the RCR recommendations by Center for Biologics Evaluation and Research and the gene therapy community. Public discussion and development of these supplemental recommendations have taken place during the Retroviral Breakout Sessions at the 1996 and 1997 FDA/NIH Gene Therapy Conferences, with representatives of the gene therapy community, and through the publication of the FDA considerations on these issues (12).

III. RECOMMENDATIONS FOR PRODUCT TESTING

A. When to Test

RCR may develop at any step during manufacturing from development of the initial master cell bank through production of the retroviral vector supernatant. In addition, the growth of ex vivo transduced cells provides the potential for amplification of any RCR contaminant which may be below the level of detection in the retroviral vector supernatant. Therefore, current testing recommendations include testing of material from multiple stages of product manufacture (see Table 1). Use of a cell bank system is recommended in order to ensure an adequate and consistent supply of vector producer cells (VPC). The Master Cell Bank (MCB) is a collection of cells of uniform composition derived from a single tissue or cell. The Working Cell Bank (WCB) is derived from one or more ampules of the MCB, expanded by serial subculture to a specified passage number (refer to Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, 1993).

1. Testing of Vector Producer Cell Master Cell Bank (one time testing)

   Both VPC and supernatant from production of a MCB should be tested for RCR using a cell line permissive for the RCR most likely to be generated in a given producer cell line. For example, VPC containing amphotropic Murine Leukemia Virus (MLV) envelope should be tested for RCR on a cell line such as *Mus dunni* that is permissive to infection by amphotropic MLV-like RCR, while VPC containing the gibbon ape leukemia virus envelope should be tested on a human cell line. Other retroviral envelopes should be tested on a cell line permissive for infection by the relevant RCR.

If derivation of VPC includes use of a retroviral vector containing an envelope distinct from the packaging vector, for example, an ecotropic MLV, the potential exists for introduction of an RCR with that envelope. Even though an ecotropic MLV RCR may present a minimal direct safety risk to humans, the presence of any replication-competent genome in the VPC is problematic because of the increased probability of generating an RCR with a human host range by recombination with elements within the VPC.

In those cases where VPC are derived at any step by infection with an ecotropic retroviral vector, testing of the MCB for the presence of ecotropic RCR is recommended. Both cells and supernatants should be tested using a method validated to detect the appropriate positive control (for example: D56 (2) or XC (10)). Refer to the guidance provided in section III.B. to determine the amount of material for testing.

2. Working Cell Bank Testing (one time testing)

Either supernatant testing or cocultivation of cells for RCR is recommended using conditions described for master cell bank testing.

3. Testing of Retroviral Vector Supernatant Product and End of Production Cells

Both retroviral vector supernatant lots and end of production cells should be tested for RCR as specified in section III.B. This recommendation is based on data and experience reported at the 1997 FDA/NIH Gene Therapy Conference in which RCR in vector production lots was not consistently detected by both assays or one assay to the exclusion of the other. These data support the position that dual testing provides a complementary approach to assuring RCR free retroviral supernatant.

4. Testing of Ex Vivo Transduced Cells

a. Cultured < 4 days after transduction

Data presented at the 1997 FDA/NIH Gene Therapy Conference indicated that for ex vivo transduced cells, a minimum culture period of 4 days from the start of transduction is necessary for amplification and detection of an RCR. As a result, for ex vivo transduced cells cultured for a period less than four days, archiving of the quantity of product needed to perform RCR testing is recommended in place of active RCR

testing. Refer to the guidance in section III.B. to determine the amount of material to be archived. Samples should be archived with appropriate safeguards to ensure long-term storage (e.g., a monitored freezer alarm storage system) and an efficient system for the prompt linkage and retrieval of the stored samples with the medical records of the patient and the production lot records.

b. Cultured $\geq$ 4 days

When ex vivo transduced cells are in culture for a period of time greater than or equal to 4 days from the start of transduction, cells and the appropriate volume of culture supernatant should be tested for RCR. Refer to guidance in section III.B. to determine amount of material for testing. In situations where ex vivo transduced cells cannot be cryopreserved during testing, and must be administered to patients prior to the availability of testing results, culture assays should be initiated at the time of patient administration. In these situations, alternative methods such as PCR may be appropriate to provide an initial analysis. Any alternative methods should be developed in consultation with CBER. Data on sensitivity, specificity and reproducibility will be needed to support the use of alternative methods.

Table 1. Recommendations for Product Testing

	RCR Testing for Expected RCR[1]		**RCR Testing for Ecotropic MLV**	
Manufacturing Step	**Cells**	**Supernatant**	**Cells**	**Supernatant**
MCB				
-Derived by infection with Ecotropic MLV vector	**Yes**	**Yes**	**Yes**	**Yes**
-Derived by transfection of retroviral vector plasmid	**Yes**	**Yes**	No	No
WCB	**Yes OR**	**Yes**	No	No
End of Production Cells	**Yes**	NA[2]	No	No
Vector-Containing Supernatant	NA	**Yes**	No	No
Ex vivo Transduced Cells				
-Cultured <4 days after transduction	No - archive	No - archive	No	No
-Cultured ≥ 4 days after transduction	**Yes**	**Yes**	No	No

[1]RCR testing should be based on the type of packaging cell line used to derive the VPC. Consult text, section III.A.1. for details.
[2]NA, not applicable

B. Amounts for Testing

1. Supernatant Testing

In all cases, 5% of the total supernatant may be tested by amplification on a permissive cell line. In instances where supernatant production volumes are greater than 6 liters, an alternative approach as described below may be applied. In order to utilize the alternative approach, the largest volume where a single infectious RCR can be detected must first be determined. When high titer retroviral vector preparations are used, interference in RCR detection may occur. In such cases, detection of a single RCR may require use of such small volumes in each test that the application of this alternative approach may not be practical. Sponsors are encouraged to develop more sensitive detection methods that overcome the interference effect of high titer retroviral vector preparations in order to use the alternative approach.

a. Alternative approach for determining total volume of retroviral vector supernatant to be tested

A statistical approach has been applied to the determination of the total volume of retroviral supernatant to be tested for RCR. This calculation is independent of production lot size and is based on the application of the Poisson distribution. It is recommended that sufficient supernatant be tested to ensure a 95% probability of detection of RCR if present at a concentration of 1 RCR/100 ml. At this concentration, a volume of about 300 ml will have a 95% probability of containing an RCR. Therefore, assuming the assay is sensitive enough to detect a single RCR, a test volume of 300 ml will provide 95% probability of detecting RCR. A more detailed explanation of the rationale and the mathematical formulas applied is found in Appendix 1-1.

To support the underlying assumption that a single retrovirus will be detected, one must determine a volume in which a single RCR can be detected by an individual RCR assay. Based on the determination of this volume, the total test volume should then be divided into replicate samples, each containing the volume demonstrated to detect a single RCR. An RCR standard has been developed, its infectious titer has been determined, and it is available through the American Type Culture Collection (ATCC). The standard can be used as a reference for determination of the volume in which a single RCR can be determined.

Refer to Appendix sections 1-2 and 1-3 for detailed information about the RCR standard and how it can be used to determine the replicate size and number for RCR detection.

b. Assays for supernatant testing

Supernatant assays should include culture of supernatant on a permissive cell line [ex. *Mus dunni* for amphotropic MLV (5)] for a minimum of *5* passages in order to amplify any potential RCR present. The amplified material may then be detected in an appropriate indicator cell assay [e.g., PG-4 S^+L^- (1)]. All assays should include relevant positive and negative controls to assess specificity, sensitivity and reproducibility of the detection method employed. Each lot of retroviral vector supernatant should be tested for inhibitory effects on detection of RCR by using positive control samples that are diluted in vector supernatant.

2. Cell Testing

The current recommendation to test 1% of the total cells or 10^8 (whichever is less) pooled vector-producing cells or ex vivo transduced cells by co-culture with a permissive cell line will remain in place. Public consensus expressed at the 1996 and 1997 FDA/NIH Gene Therapy Conferences was in support of the current recommendations for cell testing, in light of the variety of vector producing cells and vector backbones used, and the difficulty that is presented in development of a standard RCR producing positive cell stock.

Co-culture assays should include culture with a permissive cell line [ex. *Mus dunni* for amphotropic MLV (5)] for a minimum of five passages in order to amplify any potential RCR present. The amplified material may then be detected in an appropriate indicator cell assay [e.g., PG-4 S+L- (1)]. All assays should include relevant positive and negative controls to assess specificity, sensitivity and reproducibility of the detection method employed.

IV. RECOMMENDATIONS FOR PATIENT MONITORING

Active monitoring for evidence of RCR infection in patients enrolled in gene therapy clinical trials using retroviral vectors is currently recommended in a letter to Sponsors of INDs Using Retroviral Vectors, dated September 20, 1993. Based on input from the gene therapy community, problematic aspects of the current recommendations were defined as

the number of time points for testing, the requirement for life-long annual testing, and the types of assays recommended.

A. Testing Schedule

This guidance presents alternatives to the time points for monitoring originally described in a letter to Sponsors of INDs Using Retroviral Vectors, dated September 20, 1993. These recommendations are based on data accumulated in on-going gene therapy clinical trials using retroviral vectors (6, 7). The monitoring schedule recommended here should include analysis of patient samples at the following time points: pre-treatment, 3 months, 6 months, 1 year after treatment, and yearly thereafter. If all post-treatment assays are negative during the first year, the yearly samples should be archived. Samples should be archived with appropriate safeguards to ensure long-term storage (e.g., a monitored freezer alarm storage system) and an efficient system for the prompt linkage and retrieval of the stored samples with the medical records of the patient and the production lot records.

If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up should be undertaken, in consultation with CBER. It is further recommended at the time of collection of the yearly patient specimen, that a brief clinical history should be obtained. This history should be targeted towards determination of clinical outcomes suggestive of retroviral disease, such as cancer, neurologic disorders, or other hematologic disorders. Suspect clinical outcomes may trigger additional analysis of archived samples, in consultation with CBER. If patients die or develop neoplasms during a gene therapy trial, every effort should be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue.

B. Recommended Assays

Two methods are currently in use and recommended for detecting evidence of RCR infection in patients: 1) detection of RCR-specific antibodies; and 2) analysis of patient peripheral blood mononuclear cells by polymerase chain reaction (PCR) for RCR-specific DNA sequences. The choice of assay may depend on the mode of vector administration and the clinical indication. For example, it has been shown that direct administration of VPC or repeat direct injection of a vector can result in vector-specific antibodies which do not correlate with the presence of RCR (6, 7). Therefore, in cases where vector or vector-producing cells are directly administered, a PCR assay may be preferable over serologic monitoring. Additional instances where monitoring of patients by PCR may be preferred over serologic monitoring, are those cases where the patients

are immunocompromised to an extent that antibody production may be minimal or not at all. In either situation, all positive results should be pursued by direct culture assay to obtain and characterize the infectious viral isolate.

V. DOCUMENTATION OF RCR TESTING RESULTS

RCR testing results from production lots and patient monitoring should be documented in an amendment to the investigational new drug (IND). Positive results from patient monitoring should be reported immediately as an adverse experience in the form of an IND safety report (21 CFR 312.32). Negative results should be reported by way of the IND annual report (21 CFR 312.33). In addition, CBER encourages members of the gene therapy community to publish data and/or provide permission in the IND for FDA to discuss data publicly in order to enhance the cumulative data base on RCR testing assays, experience with different vector producer cell lines, patient monitoring and safety.

VI. CONCLUSION

This guidance provides additional guidance for testing for RCR associated with the use of gene therapy retroviral vectors. These supplemental recommendations are based on data and analyses generated by CBER and by members of the gene therapy community. For safety testing of retroviral vectors or vector-transduced cells, IND sponsors may either follow the recommendations previously provided in the "Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy," or follow the recommendations outlined here. Application of this supplemental guidance: 1) effectively reduces the volume of supernatant required for testing, especially in the case of large volume retroviral supernatant production lots; 2) revises the time points tested and types of assays which should be used to monitor patients who are treated in gene therapy clinical trials which involve the use of retroviral vectors; and 3) changes the recommendation for life-long monitoring from active monitoring on an annual basis to collection and archiving of patient samples and tracking of relevant clinical history on an annual basis.

A retroviral vector supernatant standard has been developed to aid in measurement of assay sensitivity. Availability of this standard supports the use of a statistical approach for determination of volume of retroviral supernatant to be tested. In addition, the retroviral supernatant standard will provide a tool for comparing the sensitivity of RCR detection by different labs and/or testing methods and may lead to improvements in assay sensitivity.

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APPENDIX

1-1. Derivation of Recommendation for Test Volume for RCR Detection

Assuming the RCR are present in the production lot at a concentration (**c**) and that an assay will detect a single retrovirus in the sample, the probability (**p**) of detecting retrovirus in a volume ($\mathbf{V_t}$) is given by the formula: $\mathbf{p= 1-exp(-cV_t)}$, because the number of RCR in V_t follows a Poisson distribution with a parameter $\mathbf{cV_t}$. Solving for $\mathbf{V_t}$, one gets the following equation:

$$\mathbf{V_t= - (1/c) \ln (1-p),}$$

where **ln** denotes the natural logarithm.

Value for p

For the use of this formula, it is recommended that the value for **p** be set at 0.95. With the recommended replicate size and number defined in Appendix 1-3, **p** becomes the probability of detecting an RCR in the production lot.

Value for c

It is recommended that the value for **c** be set no higher than 0.01 RCR/ml or 1 RCR/ 100 ml. If the concentration of RCR in the production lot is 0.01 RCR/ml or greater, then the probability of detection is at least 0.95. If the production lot contains RCR at a concentration of <0.01 RCR/ml, the RCR may not be detected and would be administered to the patient.

Value for V_t

With the recommended value for **p** and **c**, the total volume of retroviral supernatant to be tested, independent of lot size, is calculated as follows:

$$\mathbf{Vt = - (1 / 0.01\ RCR/ml) \ln (1 -0.95) @\ 300\ ml}$$

Proposals to use smaller volumes should be developed and reviewed in consultation with CBER.

1-2. Empirical Determination of Assay Sensitivity

In collaboration with the ATCC, a standard retroviral stock (ATCC # VR-1450) has been established for use in determination of sensitivity and validation of assays used to detect the presence of replication competent retrovirus which would be

produced from VPC containing amphotropic envelope. This stock can be used to determine the relative assay sensitivity for detecting RCR. This information can subsequently be used to determine the size of replicates of retroviral supernatant to be tested that will ensure detection of a single retrovirus and thus, the number of replicates to ensure an adequate total volume, V_t, as specified in this guidance (see Appendix 1-3). The virus stock is derived from a cell line which has been transfected with a molecular clone encoding Moloney murine leukemia virus (MoMLV) with a substitution of the envelope coding region from the 4070A strain of amphotropic murine leukemia virus (A-MLV) (7). Therefore, this virus stock represents a typical recombinant virus that could be generated in a retroviral packaging cell line containing coding sequences for a MLV envelope. The infectious titer of the viral stock prepared by ATCC was determined using the direct S^+L^- PG-4 assay (1). The stock was independently assessed for infectious titer by several different laboratories. The result of this analysis established the infectious titer +/- SD of the first lot of virus stock to be 6.9×10^7/ml (standard deviation for three experiments is 2.0×10^7/ml). Thawing and refreezing of the material appeared to result in a lower detectable infectious titer of 3.7×10^6/ml (standard deviation of 4.7×10^6/ml). Periodically, the vector stock will be replenished and the infectious titer of the new stock evaluated in comparison to the first lot.

The standard virus stock and its infectious titer can be used as a positive control to empirically determine the relative sensitivity of assay methods used for detection of RCR in retroviral vectors. In particular, this stock will allow investigators to determine the largest test volume in which a single RCR can be detected. The determination should be performed in the presence of a retroviral vector supernatant typical of a production lot in order to control for inhibitory effects of the retroviral vector particles on detection of RCR. Availability of this standard should allow individual investigators to establish this methodology in their own laboratories, as well as allow exploration of alternative methods for detection of RCR.

1-3. Formula to Determine Replicate Size and Number

Depending on the volume in which a single RCR can be detected by an individual RCR assay (as determined by use of the RCR standard, Appendix 1-2), it may be necessary to divide the total test volume into several replicate samples to ensure the detection of RCR in the sample. The number of replicates (**r**), can be determined using the formula,

$$\mathbf{r = V_t / V_s},$$

where $\mathbf{V_s}$ is the volume in which one RCR can be consistently detected (see Appendix

1-2 for determination of $\mathbf{V_s}$). For example, if 1 RCR can be detected in 2 ml, then the total test volume of 300 ml may be tested in 300/2 = 150 replicates of volume $\mathbf{V_s}$ or 150 2-ml replicates.